Physiology & Behavior, Vol. 23, pp. 1163–1164. Pergamon Press and Brain Research Publ., 1979. Printed in the U.S.A.

Thyroid and Thymus Morphology in Rats Following Sudden DRL Reinforcement Schedule Changes[1]

RICHARD M. KREMA,[2] MICHAEL A. PERSINGER[3] AND VICTOR SARTOR[4]

Behavioral Neurobiology Lab, Department of Psychology, Laurentian University, Sudbury, Ontario, Canada, P3E 2C6

Received 7 November 1978

KREMA, R. M., M. A. PERSINGER AND V. SARTOR. *Thyroid and thymus morphology in rats following sudden DRL reinforcement schedule changes.* PHYSIOL. BEHAV. 23(6) 1163–1164, 1979.—The possibility that sudden and maintained changes in an operant reinforcement schedule could induce discernable alterations in tissue morphology was explored. Numbers of thyroid follicles, thyroid (perifollicular) mast cells and thymus mast cells were counted from rats that had been adjusted to 80% free-feed weight and either shifted and maintained upon a DRL 12 sec schedule for 1 to 7 days after two weeks of DRL 6 sec training (DRL 6/12 sec group) or maintained on the DRL 6 sec condition (DRL 6 sec controls). Except for a marginal ($p=0.06$) decrease in thyroid mast cell numbers for the DRL 6/12 sec group, relative to the DRL 6 sec controls, these usually responsive morphological measures did not demonstrate statistically significant alterations.

Histology Mast cells DRL Thyroid Rats Thyroid follicles Thymus Operant schedule

BIOLOGICAL alterations following relatively sudden and maintained shifts in reinforcement schedules of life style changes, e.g., death of spouse, promotion, divorce, have been measured or implied in a variety of recent studies involving human subjects [4,5]. In rats, sudden and maintained shifts in reinforcement schedules such as CRF (continuous reinforcement), extinction or changing a FR (fixed ratio) 50 to FR 125, are associated with elevations in plasma corticosterone [1] and eosinopenia [6] within an hour. More long-termed alterations, such as elevations in relative blood neutrophil numbers, have been reported for at least seven days after sudden shifts from FR 20 to FR 40 or FR 60 [12], or from DRL (differential reinforcement of low rates of responding) 6 sec to DRL 12 sec [10] schedules.

We decided to explore the extent to which changes in these non-electric shock associated reinforcement schedules could alter organismic function by determining whether any histologically discernable alterations in tissue occurred. Two tissues were selected for analysis: the thyroid and thymus. The thyroid is associated with general metabolic rate, grossly reflected in follicle numbers or size, while the thymus is intimately involved with immune responses. Both systems have been implicated in diseases/disorders associated with changes in life styles or reinforcement schedules.

Although both organs contain copious numbers of indigenous cells, we required a cytological element, existing in experimentally tractable numbers, that reflected the activity of the organs. The mast cell (MC) which can be easily and clearly discriminated because of its metachromatic response to certain stains, occurs at experimentally reasonable numbers within these tissue. MC numbers in the thyroid are associated with levels of TSH [3], and are correlated with follicle numbers [9] while in the thymus MC numbers respond to levels of corticosteroids [2]. In the present study, we determined whether: (1) thyroid follicle, (2) thyroid MC and (3) thymus MC, numbers were altered in rats that had been shifted from a DRL 6 sec to a DRL 12 sec reinforcement schedule.

The thyroids and thymuses of 40, 130 day old Wistar rats (*Rattus norvegicus*) whose behavioral and blood measures have been reported previously [10] were used in this study. Thirty six of the rats had been food deprived over an 8-day period and then maintained at 80% free-feed body weight during the entire experiment while the remaining 4 rats were used as ad lib references. After two weeks of daily 30 min DRL 6 sec training, 24 of the 36 rats were shifted to a DRL 12 sec contingency. In this situation, the subjects (DRL change group) were required to inhibit lever pressing behavior for at least 12 sec in order to receive 35 mg Noyes food pellet reinforcement, instead of the well learned 6 sec period; this sudden alteration is associated with significant perturbations

[1]This work was supported by Canadian National Research Council Grant Number A0291
[2]Now at: Neuroscience Department, McMaster University, Hamilton, Ontario, Canada
[3]Thanks to Gyslaine Lafrenière for histological assistance. Send reprint requests to: Dr. M. A. Persinger, address above.
[4]Now at: Department of Psychology, Guelph University, Guelph, Ontario, Canada

Copyright © 1979 Brain Research Publications Inc.—0031-9384/79/121163-02$02.00/0

TABLE 1

MEANS AND STANDARD DEVIATIONS FOR NUMBERS (PER mm^3) OF THYMUS MAST CELLS, THYROID MAST CELLS AND THYROID FOLLICLES FROM RATS THAT HAD BEEN SHIFTED FROM DRL 6 SEC TO DRL 12 SEC (DRL 6/12 SEC), MAINTAINED ON DRL 6 SEC (DRL 6 SEC CONTROLS) OR CONTINUED IN A NON-TESTING, NON-FOOD DEPRIVED SITUATION (AD LIB REFERENCES)

Numbers ($\times 10^3/mm^3$)	DRL 6/12 sec (n=24)	DRL 6 sec Controls (n=12)	Ad Lib References (n=4)
Thymus MCs	37.9 ± 9.4	46.0 ± 16.9	39.8 ± 11.7
Thyroid MCs	12.4 ± 1.8[b]*	13.7 ± 2.1[a]	10.8 ± 1.2[c]
Thyroid follicles	27.3 ± 2.9	27.8 ± 2.3	28.6 ± 0.8

*a vs b, $p<0.10$.
a vs c, $p<0.05$.

in emotional and operant behavior [7]. The remaining 12 rats were maintained on the DRL 6 sec schedule (DRL controls) for the entire experiment. A total of six rats from the DRL change group were killed at each of the following times: (1) within 30 min of the change, (2) 1 day after the change, (3) 3 days after the change (3 days of DRL 12 sec experience) and (4) 7 days after the change (7 days of DRL 12 sec experience). A total of 3 DRL control rats were killed at each of these times. Specific procedural details and other control features of this experiment have been reported [10]. Thymus and thyroid tissues had been fixed in ethanol-formalin-acetic acid, processed, paraffin embedded and sectioned at 6 μm. The numbers of thyroid follicles and MCs (as defined by both metachromasia and morphology), were determined for each rat's thymus and thyroid tissues according to Persinger, *et al.* [8,9].Analysis of variance and other statistics were completed by computer.

The means and standard deviations for numbers of thymus MCs, thyroid MCs and thyroid follicles per mm^3 of tissue for rats that had been shifted from a DRL 6 sec schedule to a DRL 12 sec (DRL 6/12 sec), maintained on the DRL 6 sec schedule (DRL 6 sec controls) or never tested and allowed food ad lib are shown in Table 1. Two way analyses of variance according to main effects: DRL 6/12 sec vs DRL 6 sec control and post-step kill time: Day 0, Day +1, Day +3 and Day +7, demonstrated no large significant main effects or interactions for any of the measures. However, the thyroid MC difference between the DRL 6/12 sec group and DRL 6 sec controls showed marginal statistical significance, $F(1,30)=3.83$, $p=0.06$. Ad hoc one way analysis of variance between DRL 6/12 sec, DRL 6 sec controls and ad lib references was significant, $F(2,37)=4.13$, $p<0.05$, for the thyroid MC numbers only. Scheffe's test set at $p<0.05$ indicated that the major difference existed between the DRL 6 sec group and the ad lib controls.

Our results are somewhat surprising in light of the typical response of these cells and of thyroid follicles to relatively low level stimulation, such as the differences in caging conditions [8,11]. On the bases of these histological studies and hematological measures [10], we expected much larger effects from the autonomically disruptive consequences of DRL shifts [7]. The possibility that schedule shifts reported to evoke more intense and maintained physiological changes, such as shifts from FR 20 to FR 60 [12] could produce clear morphological consequences must still be evaluated.

REFERENCES

1. Coover, G. D., L. Goldman and S. Levine. Plasma corticoterone increases produced by extinction of operant behavior in rats. *Physiol. Behav.* **6:** 261–263, 1971.
2. Csaba, G. *Regulation of Mast Cell Formation.* Budapest: Akademiai Kiado, 1972.
3. Ericson, L. E., R. Hakanson, A. Melander and R. Sundler. TSH-induced release of 5-hydroxytryptamine and histamine from rat thyroid mast cells. *Endrocrinology* **90:** 795–801, 1972.
4. Fredrick, J. F. Grief as a disease process. *Omega.* **7:** 295–306, 1977.
5. Holmes, T. H. and M. Masuda. Life changes and illness susceptibility. In: *Separation and Depression.* edited by J. P. Scott and E. C. Senay. Washington, D. C.: American Association for the Advancement of Science, 1973.
6. Persinger, M. A., K. P. Ossenkopp, V. Kamaya and J. J. Pear. Physiological changes associated with changing fixed ratio schedules in rats. *Psychol. Rep.* **35:** 847–850, 1974.
7. Persinger, M. A., G. F. Lafrenière and H. Falter. Oral taurine effects on inhibitory behavior: response transients to step-like schedule changes. *Psychopharmacology* **49:** 249–252, 1976.
8. Persinger, M. A. and D. J. Coderre. Thymus mast cell numbers following perinatal and adult exposures to low intensity, 0.5 Hz magnetic fields. *Int. J. Biometeor.* **22:** 123–128, 1978.
9. Persinger, M. A., F. G. Lafrenière and N. J. Carrey. Thyroid morphology and wet organ weight changes in rats exposed to different low intensity 0.5 Hz magnetic fields and pre-experimental caging conditions *Int. J. Biometeor.* **22:** 67–73, 1978.
10. Persinger, M. A., N. J. Carrey, G. F. Lafrenière and A. Mazzuchin. Step-like DRL schedule change effects on blood chemistry, leukocytes and tissue in rats. *Physiol. Behav.* **21:** 899–904, 1978.
11. Persinger, M. A., N. J. Carrey, G. F. Lafrenière, and A. Mazzuchin. Thirty-eight blood, tissue and consumptive measures from rats exposed perinatally and as adults to 0.5 Hz magnetic fields. *Int. J. Biometeor.* **22:** 213–226, 1978.
12. Valliant, P. M. The effects of FR schedule shifts on physiochemical parameters of the albino rats. Unpublished dissertation. University of Windsor, Windsor, Ontario, Canada, 1978.